

ENVIRONMENT AND TRICHOME MORPHOGENESIS IN NICOTIANA

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Trichomes of *Nicotiana* have been elaborately studied (reviewed by Goodspeed, 1954) but employment of their descriptions as taxonomic aids has largely been frustrated by environmentally induced variation. Hence, it became the view of the authors that these structures would be suitable objects for a study of the roles of environmental factors in morphogenesis.

Temperature, pressure, light, and, principally, mineral nutrients were experimentally varied.

MATERIALS AND METHODS

Seeds were germinated upon filter paper moistened and placed within petri dishes. Though not pretreated, the seeds were covered by a second moist filter-paper sheet for three days. Percentage of germination was high for all varieties (70 to 80 percent). Experimental treatment followed transfer to washed-sand culture.

"Flowering tobacco" seeds were obtained from several commercial houses and represented horticultural varieties within the subgenera *Tabacum* and *Petunioides*. The suppliers identified the forms as Day-light, Sensation, Evening Star, *Nicotiana alata*, Crimson Bedder and Affinis.

The seedlings were transferred from filter paper to vessels containing washed sand and the appropriate nutrient medium. Control fluid was Pfeiffer's solution (Miller, 1938). Fluid level was maintained at saturation by constant drip or, in some procedures, by periodic addition.

Every two to four days representative seedlings were removed from the culture, whole-mounted, and studied microscopically. The trichomes of the cotyledons were observed without staining, *in vivo*. Photographs and camera-lucida drawings supplemented other notation.

Temperature-varied plants were grown at 20°C and 41°C with controls at room temperature. All were bathed by Pfeiffer's nutrient solution. These plants were exposed to incandescent illumination constantly.

Pressure-varied plants were kept at 20 lbs./sq. in. in a metal container with a controlled eight-hour light period each day. Pressure was maintained by filtered air from the laboratory line. The temperature in the vessel was 38°C.

Nutrient-varied plants received Pfeiffer's solution lacking, respectively, manganese (manganese chloride), zinc (zinc chloride), boron (boric acid), and copper (copper chloride). Controls received the complete medium. The plants were grown within a glass-enclosed case

and received light from two sixty-watt incandescent bulbs at a distance of ^c1 foot for eight hours each day. Additional extraneous room light reached the plants. The average light-period temperature was 34°C; 4-8°C less during other hours.

To avoid the variation incident upon trace contamination, a single chemical supply was used and the number of nutrient solutions was limited to two.

Seedlings were also grown in garden loam and trichome development was compared with that in Pfeiffer's-medium control plants. Observations continued, in every instance, for about 40 days.

OBSERVATIONS

Trichomes developed identically on cotyledons of soil-grown and Pfeiffer's-medium sand-culture grown plants. Though from variety to variety growth patterns varied with regard to a time preference, regularity within the members of a variety was of a high order under the conditions of this study.

The developmental pattern characteristic of Affinis is represented by Figures 1-5. Other varieties were similar though maturation periods varied from 43 days in Evening Star to 23 days in Daylight.

Under the conditions of temperature, pressure, and light variation, of this investigation, no significant effect upon development of trichomes was noted.

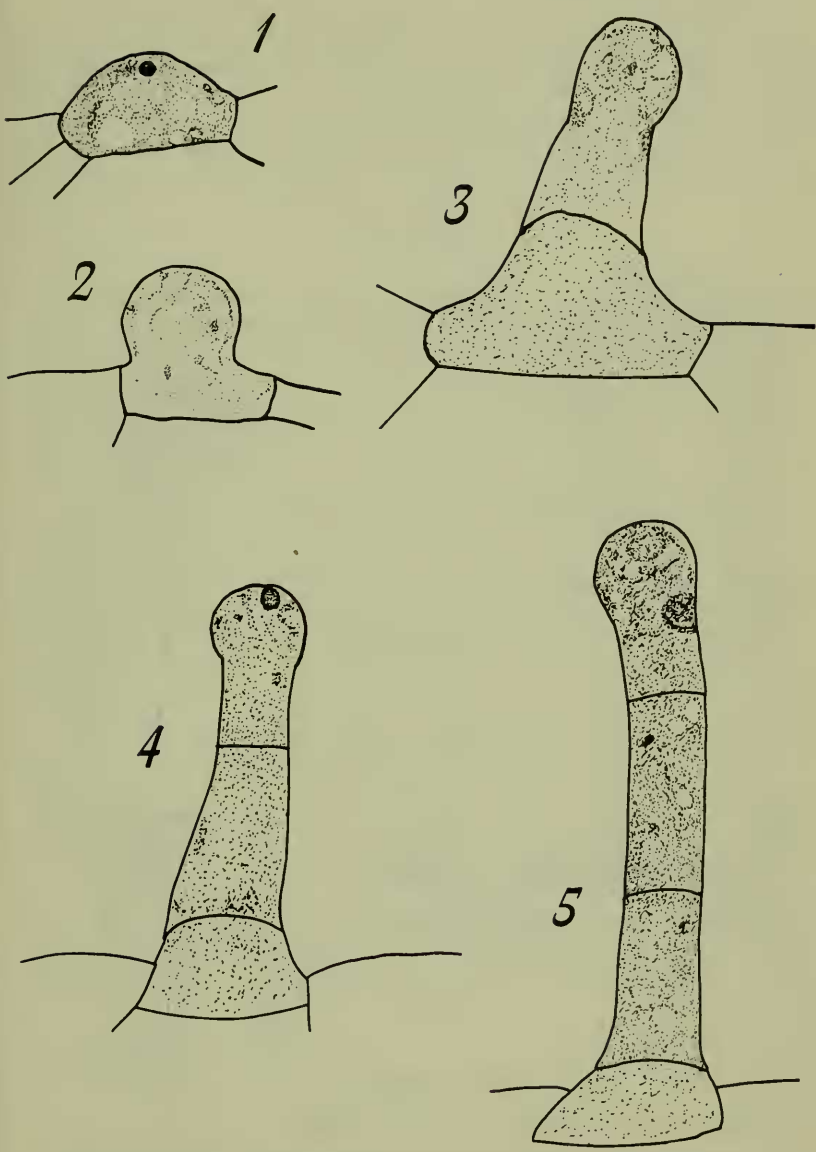
Deprivation of trace elements was reflected in trichome development. Boron deficiency, perhaps stimulatory in early development resulted in a reduction in the number of mature hairs. Copper-deprived plants matured in smaller numbers and there was frequently a modification of the normally knob-like tip to a more or less pointed apex. Manganese-deficient plants were slightly inhibited. (Only the Evening-Star variety was grown with boron, copper, and manganese deficiencies.)

TABLE I. Trichome development in control and zinc-deprived plants, of the variety Affinis, 23 days after germination. ¹

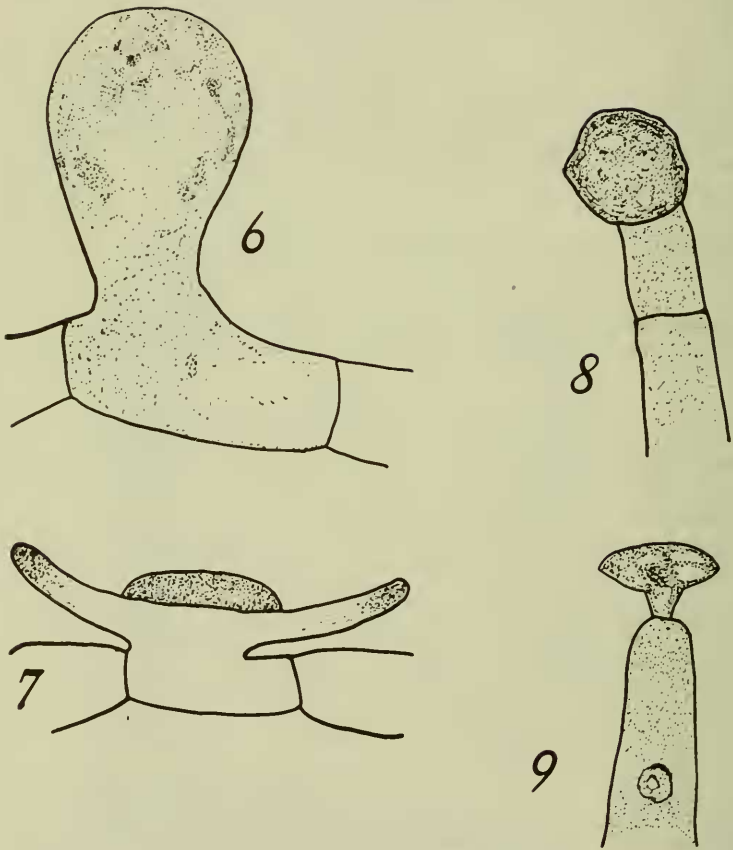
1 cell trichomes		2 cell		3 cell		4 cell		aberrant		total	
+ zinc	-	+	-	+	-	+	-	+	-	+	-
6.4	4.4	29.8	19.4	2.8	4.2	.2	.8	.8	2.6	39.8	31.4
18.1%	14.0%	74.4%	81.8%	7.0%	13.4%	.5%	2.5%	2.0%	8.3%	-	-

¹ Average per seedling along cotyledon edges.

Zinc deprivation markedly affected the development of the trichomes. Table I is a comparison of control and zinc-deprived plants in Affinis at 23 days after germination. The average total number of trichomes per leaf (edges of leaf only) is 39.8 in control plants and 31.4 in zinc-deprived plants.



FIGURES 1-5. Normal developmental pattern of cotyledon trichomes in *Affinis*. Figures 1 and 2. Earlier and later one-cell stages. Figure 3. Two-cell stage. Figure 4. Three-cell stage. Figure 5. Four-cell stage.



FIGURES 6-9. Aberrant trichomes. Figure 6. Giant one-cell hair (Affinis). Figure 7. Peltate-like structure (Evening Star). Figures 8 and 9. Degenerating apical cells; earlier and later (Affinis).

In all varieties, the most striking result of zinc deprivation is the production of monsters, degenerative, and variously aberrant forms. Evening Star showed the most extreme variation, developing lateral extensions of the basal cell and flattening of distal cells to yield a scale-like structure (Figure 7). Though such apparent mimicries were not observed in other varieties, a wide range of aberrant forms was observed in all types. Some of these are illustrated in Figures 6, 8, and 9.

DISCUSSION

The possibility exists that the morphological effects of zinc deprivation may not necessarily indicate a unique activity in hair cells as apart from other of the plant tissues. That is, these structures may be capable, presumably by virtue of their relative physical independence as compared with totally surrounded tissue cells, of structurally reflecting a general physiologic pattern. The trichomes are, in any event, a sensitive indicator.

Development of abnormal epidermal hairs, in one variety resulting in the appearance of peltate structures (not frequently found on the cotyledons) and in all varieties in the emergence of widely aberrant forms, indicates strongly that zinc is related to the normal morphogenesis of these structures. The peltate-like development of hairs under zinc deprivation is suggestive that this may be a factor in the mechanism by which normal peltate hairs have evolutionarily appeared. (Peltate hairs do, of course, commonly appendage mature *Nicotiana* leaves.)

What the relationship between morphogenesis of these structures and zinc may be is conjectural beyond the obvious likelihood that the effects observed are intimate with the function of a zinc-metalloenzyme system. Seven such systems are biologically known (Vallee, 1959) and it seems not unlikely that others may exist.

More directly, several workers have related zinc to indole acetic acid. Tsui (1948) reported, that in tomato, zinc may function enzymatically, in the synthesis of auxin. Skoog (1940) believes that zinc is not directly involved in the synthesis of auxin but rather functions to prevent its oxidative destruction.

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LITERATURE CITED

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- Vallee, Bert L. (1954) Biochemistry, physiology and pathology of zinc. Physiol. Rev. 39: 443-490.

ADDITIONAL NOTES ON THE GENUS CASTELIA. I

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These notes are supplementary to my monograph of the genus as published in Phytologia 6: 232--241 (1958).

CASTELIA Cav.

Additional synonymy: Bastelia Cav. ex Moldenke, Alph. List Cit. 4: 1088, sphalm. 1949.

Additional & emended literature: Walp., Repert. Bot. Syst. 4: 33 & 36. 1845; Hook. & Jacks., Ind. Kew. 2: 493 (1894) and 2: 1179. 1895; Sanzin, Anal. Soc. Cient. Argent. 88: 106, fig. 10. 1919; Moldenke in Fedde, Repert. 41: 10 & 62. 1936; Moldenke, Alph. List Cit. 1: 170, 230, 272, & 304 (1946), 2: 337, 338, 355, 358, 371--373, 377, 379, 381, 407, 423--426, 430, 434, 435, 438--442, 500, 537, 561, 565--567, 575, 580, 584, 593, 598--600, 613, 617, & 626--628 (1948), 3: 662, 663, 672, 673, 703, 728, 731, 732, 735--737, 746, 767, 784, 812, 838, 848, 859, 864, 865, 880, 884, 893, 900, 903, 911, 913, 931, & 940 (1949), and 4: 979, 980, 983, 1010, 1013, 1036, 1043, 1044, 1049, 1050, 1056, 1062, 1070, 1075, 1078, 1088, 1090, 1091, 1115, 1120, 1138, 1187, 1192, & 1302. 1949; Moldenke, Résumé 82, 87, 113, 120, 123, 214, 238, 249, 250, 333, 335, 340, 369, 371, 377, 407, 423, & 445. 1959; Moldenke, Résumé Suppl. 2: 8 & 12. 1960.

CASTELIA CUNEATO-OVATA CAV.

Additional synonymy: Verbena lobelioides Grah. ex Walp., Repert. Bot. Syst. 4: 33, in syn. 1845. Verbena orchiioides Hort. ex Hook. & Jacks., Ind. Kew. 2: 1179. 1895. Bastelia cuneato-ovata Cav. ex Moldenke, Alph. List Cit. 4: 1088, sphalm. 1949.

Emended illustrations: Sanzin, Anal. Soc. Cient. Argent. 88: 106, fig. 10. 1919.

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